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भाग 2 कॉपर संकुल

(दूसरा पुनरीक्षण)

Indian Standard

CHLOROPHYLL, FOOD GRADE — SPECIFICATION

PART 2 COPPER COMPLEX

(Second Revision)

ICS 67.220.20

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

June 2008 **Price Group 2**

AMENDMENT NO. 1 APRIL 2011
TO
IS 4446 (PART 2) : 2008 CHLOROPHYLL, FOOD GRADE — SPECIFICATION

PART 2 COPPER COMPLEX

(Second Revision)

(*Page* 1, *clause* **2**) — Insert the following at the appropriate place:

IS No.	*Title*
4446 (Part 1) : 2008	Chlorophyll, food grade — Specification : Part 1 Magnesium complex (*second revision*)

[*Page* 1, *Table* 1, *Sl No.* (ii), *col* 2] — Substitute 'Residual solvents (Acetone, methanol, ethanol, propan-2-ol, hexane), mg/kg, *Max*' *for* 'Residual solvents, mg/kg, *Max* Acetone, methanol, ethanol, propan-2-ol and hexane, mg/kg, *Max* (singly or in combination)[1)]' and delete footnote.

[*Page* 1, *Table* 1, *Sl No.* (ii), *col* 4] — Substitute '*D*' *for* '_'.

[*Page* 1, *Table* 1, *Sl No.* (iii), *col* 2] — Substitute 'Dichloromethane, mg/kg, *Max*' *for* 'Dichloromethane, mg/kg, *Max* [1)]' and delete footnote.

[*Page* 1, *Table* 1, *Sl No.* (iii), *col* 4] — Substitute '*D*' *for* '_'.

[*Page* 2, *clause* **5.3**(d)] — Substitute 'Net quantity' *for* 'Net content'.

(*Page* 3, *Annex* C) — Insert the following new Annex after Annex C:

ANNEX D

[*Table* 1, *Sl No.* (ii) *and* (iii)]

DETERMINATION OF RESIDUAL SOLVENTS AND DICHLOROMETHANE USING HEAD SPACE CHROMATOGRAPHY

D-0 Determine residual solvents and dichloromethane using head space chromatography as per Annex B of IS 4446 (Part 1).

(FAD 8)

Reprography Unit, BIS, New Delhi, India

FOREWORD

This Indian Standard (Part 2) (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Divisional Council.

Chlorophyll, the green pigment of plants, is extracted and widely used as a colouring matter for various food items. It readily looses its central magnesium ion under the mildest acidic conditions. Replacement of the magnesium ion with a copper ion produces a derivative, chlorophyll copper complex, from which copper ions are not removed to any appreciable extent on metabolism, thus being acceptable for colouring of foodstuffs.

This standard is one of a series of Indian Standards for natural food colours permitted under the *Prevention of Food Adulteration Rules*, 1955.

The standard was first issued in 1967 and covered the magnesium complex of chlorophyll only. It was later revised in 1986 into two parts, namely:

Part 1	Magensium complex
Part 2	Copper complex

This standard is now being revised taking into consideration the latest publication for the food colour issued by JECFA and also the latest specifications laid down under the US FDA and the EEC Directives. In this revision requirement for the purity of chlorophyll and also the limit for residual solvent has been incorporated and requirements for heavy metal contaminants made more stringent to align with the international requirements.

Requirements for chlorophyll, food grade have been prescribed under the *Prevention of Food Adulteration Rules*, 1955 and due consideration has been given to the rules in the formulation of this standard. Due consideration has also been given to the *Standard of Weights & Measures (Packaged Commodities) Rules*, 1977. However, this standard is subject to restrictions imposed under these, wherever applicable.

Description

Common Name — Chlorophyll

Synonyms — Copper chlorophyll, Copper phaeophytin, Cl Natural green 3, C.I. (1975) No. 75810; INS No. 141

C.A.S. Number — 65963-40-8

Chemical Names — The major colouring principles are:

[Phytyl (13^2R, 17S, 18S) -3-(8-ethyl-13^2-methoxycarbonyl-2,7,12,18-tetramethyl-13^1-oxo-3-vinyl-13^1 -13^2-17, 18-tetrahydrocyclopenta [at]-prophyrin-17-yl)propionate] copper (II) (Copper chlorophyll *a*].

[Phytyl (13^2R, 17S, 18S)-3-(8-ethyl-7-formyl-13^2-methoxycarbonyl-2, 12, 18-trimethyl-13^1-oxo-3-vinyl-13^1-13^2-17, 18-tetrahydro-cyclopenta [at]-prophyrin-17-yl)propionate] copper (II) (Copper chlorophyll *b*).

Chemical Formula

Copper phaeophytin *a* : $C_{55}H_{72}CuN_4O_5$

Copper phaeophytin *b* : $C_{55}H_{70}CuN_4O_6$

Molecular Weight

Copper phaeophytin *a* : 932.75

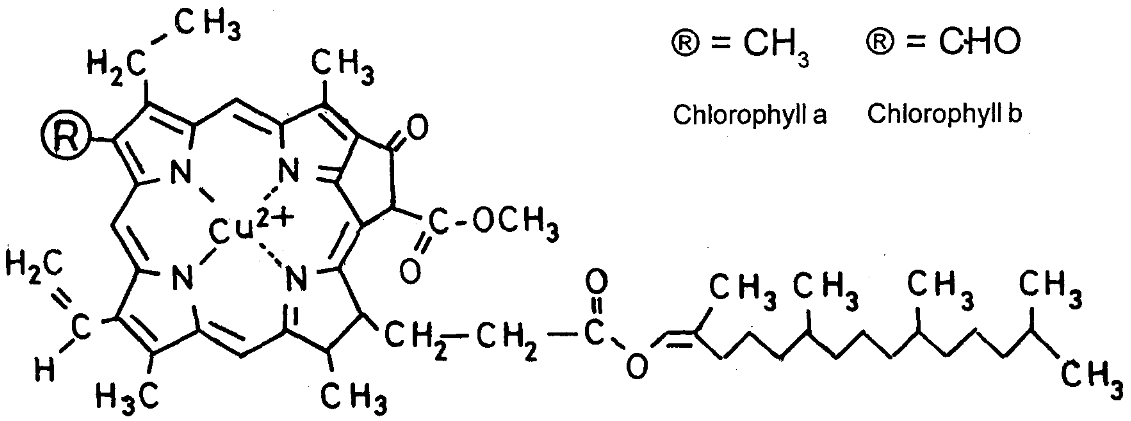
Copper phaeophytin *b* : 946.73

Solubility — Insoluble in water; soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed oils.

(*Continued on third cover*)

(*Continued from second cover*)

The structural formula of chlorophyll is given below:



Chlorophyll Copper Complex

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

CHLOROPHYLL, FOOD GRADE — SPECIFICATION

PART 2 COPPER COMPLEX

(Second Revision)

1 SCOPE

This standard (Part 2) prescribes requirements and methods of sampling and test for chlorophyll, copper complex, food grade.

2 REFERENCES

The following standards contain provisions, which through reference in this text constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1995	Methods of sampling and test for synthetic food colours (*second revision*)
2491 : 1998	Food hygiene — General principles — Code of practice (*second revision*)

3 DESCRIPTION

Chlorophyll, copper complex is a waxy solid ranging in colour from blue green to dark green depending on the source material.

4 REQUIREMENTS

4.1 Spectrophotometry

Absorbance of one percent solution of chlorophyll, copper complex (through 1-cm cell) at 422 nm in chloroform is not less than 54.

4.2 Thin-Layer Chromatography

Apply a 1 in 20 solution of the sample in chloroform as a band of the length of 2 cm to a silica 60 C plate. After drying, develop the plate by a mixture of 50 percent hexane, 45 percent chloroform and 5 percent ethanol (General purpose reagent grade chloroform is supplied with 2 percent of added ethanol as a stabilizer. The 5 percent ethanol in the solvent mixture is in addition to this), until the solvent ascends to a point 15 cm above the initial spots. Allow the solvent to evaporate, then visually examine the separated spots and identify the components of interests by their R_f values and colours.

Approximate R_f values and colour of the spots are as follows:

Copper phaeophytin *a* : 0.5, green
Copper phaeophytin *b* : 0.73, yellow/green

In addition spots may be visible for β-carotene at R_f 0.81 and xanthophyll at R_f 0.47 and 0.23.

4.3 The product shall also conform to the requirements given in Table 1.

Table 1 Requirements for Chlorophyll, Copper Complex

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex of This Standard	Clause of IS 1699
(1)	(2)	(3)	(4)	(5)
i)	Total copper phaeophytins, percent by weight, *Max*	10	A	—
ii)	Residual solvents, mg/kg, *Max* Acetone, methanol, ethanol, propan-2-ol and hexane, mg/kg, *Max* (singly or in combination) 1)	50	—	—
iii)	Dichloromethane, mg/kg, *Max* 1)	10	—	—
iv)	Free ionizable copper, mg/kg, *Max*	200	B	—
v)	Total copper, percent of total copper phaeophytins, *Max*	8	C	—
vi)	Arsenic (as As), mg/kg, *Max*	3	—	15
vii)	Lead (as Pb), mg/kg, *Max*	10	—	15

1) Method of test under preparation.

4.4 The product shall be processed, packed, stored and distributed under hygienic conditions in licenced premises (*see* IS 2491).

5 PACKING, STORAGE AND MARKING

5.1 Packing

The material shall be filled in amber-coloured glass containers or any other suitable containers under an inert gas as chlorophyll is sensitive to oxygen and light. The containers shall be such as to preclude contamination of the contents with metals or other impurities.

5.2 Storage

The material shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

5.3 Marking

Each container shall be legibly and indelibly marked with the following information:

a) Name of the material including the words 'Food Grade';
b) Name and address of the manufacturer;
c) Batch or code number;
d) Net content when packed;
e) Instruction for storage;
f) Best before .... (month and year to be given by the manufacturer); and
g) Any other requirements as given under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977 and *Prevention of Food Adulteration Act*, 1955 and Rules.

5.3.1 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

5.3.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

Representative samples of the product shall be drawn according to the method prescribed in **4** of IS 1699.

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result of analysis.

ANNEX A

[*Table* 1, *Sl No.* (i)]

METHOD FOR ASSAYING PURITY OF CHLOROPHYLL, COPPER COMPLEX

A-0 METHOD

The assaying of purity of Chlorophyll is carried out by the Spectrophotometric method.

A-1 REAGENT

A-1.1 Diethyl Ether, spectrophotometric grade.

A-2 APPARATUS

A-2.1 Spectrophotometer, capable of accurate (±1 percent or better) measurement of absorbance in the region of 350-750 nm with an effective slit width of 10 nm or less.

A-2.2 Absorption Cells, 1-cm light path.

A-3 PROCEDURE

A-3.1 Accurately weigh about 100 mg of the sample and dissolve in diethyl ether, making the volume to 100 ml. Dilute 2 ml of this solution to 25 ml with diethyl ether. The concentration of the sample should not give an absorbance at 660.4 nm that is in excess of the working range for Absorbance measurements, that is, not in excess of 0.7.

A-3.2 Measure the absorbances of the solution in a 1-cm cell against a diethyl ether blank at 667.2 nm, 654.4 nm, 649.8 nm and 628.2 nm. (These being the absorbance maxima in diethyl ether for copper phaeophytin, *a* and copper phaeophytin, *b*, respectively).

Calculate the concentration of the individual compounds in micromoles per litre from the following equation:

$$\text{Copper phaeophytin, } a = 45.6\,A\,(649.8\text{ nm}) - 2.75\,A\,(628.2\text{ nm}) + 3.10\,A\,(667.2\text{ nm}) - 35.4\,A\,(654.4\text{ nm})$$

$$\text{Copper phaeophytin, } b = 8.46A\,(649.8\text{ nm}) + 20.7\,(628.2\text{ nm}) - 1.69\,A\,(667.2\text{ nm}) + 5.13\,A\,(654.4\text{ nm})$$

Convert the figures in micromoles/litre to percentages using the following equations:

$$\text{Percent copper phaeophytin, } a = \frac{\text{Micromoles} \times 0.932\,7 \times 12.5 \times 100}{\text{Mass of sample, mg}}$$

$$\text{Percent copper phaeophytin, } b = \frac{\text{Micromoles} \times 0.946\,7 \times 12.5 \times 100}{\text{Mass of sample, mg}}$$

ANNEX B

[*Table* 1, *Sl No.* (iv)]

METHOD FOR DETERMINATION OF FREE IONIZABLE COPPER

B-1 REAGENT

B-1.1 0.5 N Hydrochloric Acid, spectrophotometric grade.

B-2 PROCEDURE

B-2.1 Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil, with the help of gentle heat. Add exactly 200 ml of water, stir mechanically and adjust the *p*H to 3.0 by careful addition of 0.5 N hydrochloric acid (avoid over-shooting).

B-2.2 Allow the above mixture to stand for 10 min. If necessary, adjust the *p*H to 3.0 by careful addition of 0.5 N hydrochloric acid. Transfer to a separating funnel and allow to stand for about 20 min. Filter the aqueous through a Whatman No. 50 filter paper, rejecting the first 10 ml.

B-2.3 Subject this solution to analysis for copper by atomic absorption spectrometry as given in **15** of IS 1699.

ANNEX C

[*Table* 1, *Sl No.* (v)]

METHOD FOR DETERMINATION OF TOTAL COPPER

C-1 REAGENTS

C-1.1 Concentrated Sulphuric Acid

C-1.2 Ten Percent (*w/w*) Hydrochloric Acid

C-2 PROCEDURE

C-2.1 Ignite about 0.1 g of the sample, accurately weighed in a silica dish, at a temperature not exceeding 500°C, until all the carbon is removed. Moisten with 1 or 2 drops of concentrated sulphuric acid and re-ash.

C-2.2 Dissolve the ash by boiling with three portions (each of 5 ml) of 10 percent (*w/w*) hydrochloric acid, filtering each addition through the same small filter paper into a 100-ml volumetric flask. Cool and make up to volume with purified water.

C-2.3 Subject this solution to analysis for copper by atomic absorption spectrometry as given in **15** of IS 1699.

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This Indian Standard has been developed from Doc : No. FAD 8 (1472).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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